

§Appl. No. 09/930,213
Amdt. dated February 24, 2004
Reply to Office Action of, October 24, 2004

IN THE SPECIFICATION:

On page 7, the first full paragraph has been amended as follows:

A subject of the invention is a nucleic acid, characterized in that it shows a differential expression in tumor cells and normal cells, comprising

- (a) one of the nucleic acid sequences shown ~~in Fig. 11~~ SEQ ID NOS 336-885;
- (b) partial sequences thereof with a length of at least 50, preferably at least 100, and especially preferably at least 200 nucleotides,
- (c) a sequence that hybridizes with a sequence that consists of (a) and/or (b) under stringent conditions, and/or
- (d) a sequence that is complementary to a sequence that consists of (a), (b) and/or (c).

On page 7, the second full paragraph has been amended as follows:

The process according to the invention makes it possible to identify genes whose expression in the Ras-transformed cells is enhanced in comparison to the non-transformed cells. These genes preferably comprise the corresponding nucleic acid sequences (T-clones) that are indicated ~~in Fig. 11~~ as SEQ ID NO 633-885 or partial sequences thereof with a length of at least 50, preferably at least 100 and especially preferably at least 200 nucleotides.

The last paragraph bridging pages 7 and 8 has been amended as follows:

In addition, the process according to the invention makes it possible to identify genes whose expression in the Ras-transformed cells is reduced in

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comparison to the non-transformed cells. These genes preferably comprise the corresponding nucleic acid sequences (N-clones) that are indicated ~~in Fig. 11~~ as SEQ ID NOS 336-632 or partial sequences thereof with a length of at least 50, preferably 100 and especially preferably at least 200 nucleotides.

On page 8, the first full paragraph has been amended as follows:

In addition to the nucleic acid sequences that are indicated in ~~Fig. 11~~ SEQ ID NOS 336-632 and partial sequences thereof, the invention also comprises nucleic acid sequences, which hybridize under stringent conditions with one of the nucleic acid sequences indicated in ~~Fig. 11~~ or partial sequences thereof (as indicated above). The term "hybridization" according to this invention is uses as in Sambrook et al., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989), 1.101-1.104. According to this invention, we thus speak of hybridization under stringent conditions, if, after washing for one hour with 1 x SSC and 0.1% SDS at 55°C, preferably at 62°C and especially preferably at 68°C, especially for 1 hour in 0.2 x SSC and 0.1% SDS at 55°C, preferably at 62°C and especially preferably at 68°C, a positive hybridization signal is observed. This invention comprises a nucleotide sequence that hybridizes under such washing conditions with a nucleotide sequence shown ~~under Fig. 11~~ SEQ IID NOS 336-885.

The last paragraph bridging pages 8 and 9 has been amended as follows:

In particular, this invention also includes genes that are homologous to the nucleic acid sequences shown in ~~Fig. 11~~ SEQ ID NOS 336-885, especially homologous genes from other species, especially human genes, or allelic

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variations of these genes. Such sequences preferably hybridize under the above-indicated conditions with the nucleic acid sequences that are shown in Fig. 11
SEQ ID NOS 336-885.

On page 17, the first full paragraph has been amended as follows:

Figure 11 (A-E) shows a correlation of nucleotide sequences (rats) (SEQ ID NOS 336-632 are N1-N297 and SEQ ID NOS 633-885 are T1-T253) to the homologous human sequences from SEQ ID NO 1-335 and the corresponding T or N clone.

On page 17, the second full paragraph has been cancelled as follows:

~~SEQ. ID NOS. 1-885; sequence SEQ. ID NOS. 1, 2, 3 . . . 335 correspond to sequences 1, 2, 3 . . . 335 according to Fig. 12. Sequences SEQ. ID NOS. 336, 337, 338 . . . 632 correspond to sequences nos. 1, 2, 3 . . . 297 according to Fig. 11, and sequences SEQ. ID NOS. 633, 634, 635 . . . 885 correspond to sequences T1, T2, T3 . . . T253 according to Fig. 11.~~

The first full paragraph bridging pages 23 and 24 has been amended as follows:

2.2 Isolation of Sequences Expressed Differentially in Transition from the Normal State to the Transformed State

cDNA clones, which preferentially represent mRNAs that are expressed in normal 208F rat cells or in transformed FE-8 cells, were obtained from two subtracted cDNA libraries. For isolation from sequences (N-clones) that are adjusted down in the transition from normal to transformed state, tester cDNA from normal 208F fibroblasts and driver cDNA from transformed FE-8 cells

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(forward-subtraction) were used. To obtain sequences (T-clones) up-adjusted during the neoplastic transformation, FE-8 cDNA was used as a tester and 208F cDNA was used as a driver (backward-subtraction). The nucleotide sequences of 1357 subtracted cDNA clones were determined after T/T cloning and bacterial transformation. In this case, 823 individual sequences were identified (Fig. 1) (Figs. 1, 11). To verify the differential expression by independent methods, subtracted cDNA sequences were amplified by PCR with use of Nested Adapter Primers. The PCR products were separated by gel-electrophoresis and transferred onto hybridization membranes. In this case, two membranes were hybridized with radiolabeled samples from normal 208F cells or transformed FE-8 cells (Reverse Northern Analysis, Fig. 6). In addition, whole RNA from 208F and FE-8 cells with Standard Northern Blots was analyzed with use of individual cDNA fragments as samples. By conventional Northern Analysis, the differential expression of 48 to 50 randomly selected cDNA fragments (96%) on Reverse Northern Blots was verified. In addition, 193 known gene fragments, which yielded no clear signals or even no hybridization signals on the Reverse Northern Membranes, were analyzed in a conventional way. The results of all Standard Northern Blot analyses with reference to differential expression of Ras-transformation targets are shown in Fig. 7. Expressed sequence tags and new sequences were not further analyzed, when the sensitivity of the Reverse Northern Blot was not sufficient to verify clearly a differential expression. The list of all differential genes classified according to selected properties of their products is shown in Fig. 2. The methods for detection of expression differences between normal and transformed makes it possible to isolate strongly and poorly expressed genes. Based on the compensating step contained in the SSH process, an identification of strongly expressed transcripts (e.g., coding for proteins of the

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cytoskeleton) and mRNAs with a low copy number (e.g., coding for transcription factors) was made possible. The cDNA fragments identified in this study represent a significant fragment of the differentially expressed genes in the two cell lines.